FINAL REPORT ON THE WEEVIL RELEASE STUDY FOR INDIANA LAKES

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INTRODUCTION

In the summer of 2000 DNR, Lake and River Enhancement Program awarded a grant to Robin Scribailo of Aquatic Restoration Systems to study the impact of milfoil weevil release on Eurasian milfoil populations in three Indiana lakes. This study was initiated because of the great interest in Indiana by lake associations interested in more effective long-term control of Eurasian milfoil. Biological control has the distinct advantage of offering control in perpetuity without the obtrusive and potentially harmful side effects of herbicide treatment.

Despite an extensive documentation on the ability of the milfoil weevil to destroy Eurasian milfoil in laboratory tests (see bibliography) and a largely anecdotal ecological literature that shows a tenuous correlation among North American lakes with populations of the milfoil weevil and declines in the coverage extent of Eurasian milfoil (Creed 1998, Creed and Sheldon, 1995) there are no quantitative ecological studies directly showing that the aforementioned correlation represents cause and effect.

For basic background papers on the life-cycle of the weevil the reader is referred to the numerous papers by Sheldon. An excellent summary of current research on the milfoil weevil can be found at the website of Newman (http://www.fw.umn.edu/research/milfoil/milfoilbc.html) An extensive milfoil bibliography is attached to the end of this report.

Three lakes were chosen for this study based on interest by the corresponding lake associations. The lakes involved are Round Lake in Whitley County, Little Turkey Lake in LaGrange County and Griffy Lake in Monroe County (Fig. 1).

Specific goals of the project were as follows;

1. To document existing conditions at the lakes in terms of the diversity and abundance of native aquatic plant populations.
2. To map the extent of existing milfoil populations and document changes over the duration of the study.
3. To quantitatively assess the impact of introduced milfoil weevils on the growth of Eurasian milfoil.
4. To assess the effectiveness of milfoil weevils as a biocontrol agent for Eurasian milfoil in the state of Indiana.

METHODS

Aquatic Plant Species Inventory

Each lake was initially surveyed to assess the nature of existing plant populations. Griffy Lake was visited on May 11, Round Lake on May 26th and Little Turkey on May 27th. These lakes were revisited respectively on June 22nd, June 16th and June 17th. We were particularly interested in documenting the existence of rare state-listed species. Lakes were surveyed from a Jon boat and by wading the entire littoral zone of each lake. In deeper water lakes were either sampled with a long-handled rake or with the use of SCUBA. Vouchers have been placed in the Aquatic Plant Herbarium of Purdue University North Central.

During the subsequent two years of this study several additional species were added to the aquatic plant species inventory lists. Changes in the distribution of species throughout the study are described in the discussion of this report. All visits in subsequent years were made within one week of the date of the original visit in the year 2000. Attempts were made to keep visitation dates as close as possible to those for the first year so as to minimize seasonal fluctuations in temperature and other environmental variables.

Mapping of Milfoil Populations

All milfoil beds were mapped using high-resolution sub-meter accuracy GPS on the second lake visit discussed above. A Trimble Pathfinder GPS unit was linked for data entry to a Casio palmtop computer. This data was then downloaded to ArcView 3.2 GIS for mapping purposes. Results for the three lakes are shown as polygon overlays onto aerial photographs color-coded for the three years (Fig. 2-4). Mapping of milfoil beds was not possible each year at each site because of specific problems encountered that are described in detail within the discussion.

Quantification of Milfoil Growth

In order to assess the true impact of milfoil weevils on Eurasian milfoil beds it was important to design an experiment that would allow an unbiased assessment of changes in both population parameters of Eurasian milfoil as well as those of other aquatic plants. Three permanent 50 meter transects were placed at Round Lake and two were placed at Little Turkey Lake on the second lake visit (Fig. 2,3).

No permanent transects were placed at Griffy Lake because milfoil populations had already senesced by the date of weevil stocking at the time of the second visit. The early senescence was an unusual occurrence that was unexpected so early in the season. A very warm spring may have contributed to an acceleration in the life-cycle of milfoil plants. Plants were also very negatively impacted by massive filamentous algae growth that caused them to sink. The algae growth may have been increased by warm temperatures and a period of heavy rains which would have released extensive nutrients into the watershed of Griffy lake. Since it was not possible to run transects in the first year at Griffy Lake none were placed in subsequent years. Analysis consisted only of random quadrats at two locations to assess biomass. The milfoil was also mapped in subsequent years.

Transects at Round Lake and Little Turkey Lake were run parallel to the shoreline through the approximate middle of the milfoil beds. Submersible 0.5 meter sq quadrats was placed either to the right or left of the transect line at 5 meter intervals. Five were chosen in each direction by randomly picking for either right or left at each 5 meter interval. Distance out from the transect was assigned from a random number table as long as it fell within the mifoil bed. The transect and each quadrat location was mapped using GPS so that they could be relocated precisely the following year. A submerged PVC post was driven into the lake bottom to mark the Northwest corner of each quadrat location.

The following parameters were measured for each quadrat:

1. Number of mifoil stems
2. Maximum height of milfoil plants

In addition to the permanent quadrats discussed above five random quadrats were destructively sampled by taking all plant material present from the 0.5 meter sq. area. These plants, in the case of Round Lake only, were analyzed as detailed below, for weevil abundance and damage. The milfoil plants were acid treated to remove marl deposits and were then dried at 100 C to obtain a dry-weight biomass.

Plants were placed in a muffle furnace at 500 ^{0} C for five hours, allowed to cool and immediately placed in a dessicator prior to weighing to generate a value for ash-free dry weight. The ash-free dry weight is the difference between the dry weight and the ash weight. The ash weight represents minerals left after all organic matter has burnt off. Subtracting the two thus is a representation of true organic matter whereas biomass includes water content and ash content.

Weevil Stocking

Weevil stocking was performed by Enviroscience of Ohio. Nine thousand weevils were stocked at each lake with three thousand being placed at each of three locations. Bundles of milfoil with weevils were attached to existing plants in milfoil beds at the lakes (Enviroscience 1997). Transect locations correspond to locations of weevil stocking. A goal of the study was to examine rates of spreading of weevils by having them stocked at the beginning of each transect and documenting damage at increasing distances from the stocking location over time. Unfortunately weevils were mistakenly stocked in the middle of the two transects at Little Turkey Lake.

Existing Weevil Populations

During initial reconnaissance of Round Lake for aquatic plant surveying damage was noted to shoot tips of milfoil. Examination of a number of shoot tips revealed the presence of a natural population of milfoil weevils. Since it would not be possible to ascertain damage caused by released weevils unless the size of the original weevil population was known efforts were made to quantify this parameter.

Plants were examined from the five biomass quadrats discussed above to determine the extent of existing weevil populations at Round Lake. The number of adult weevils and larvae were counted on each milfoil stem within the quadrats. Adult weevils are typically found in the apical meristems (shoot tips) of the plants and are highly visible. Larvae are harder to see although the first clue to their presence is usually an entry hole into the stem. A summary of this data is presented in Figure 4.

RESULTS AND DISCUSSION

Of the three lakes studied Round Lake has the greatest species richness with 28 species of aquatic plants followed by Griffy Lake with 20 and Little Turkey with 12 species. Of these species four are state-listed taxa from Round Lake (*Myosotis scorpioides, Potamogeton pusillus, P. praelongus, P. richardsonii*) while three are state-listed from Griffy Lake (*Myosostis scorpioides, Potamogeton pusillus, Zannichellia palustris*) and one is ranked from Little Turkey Lake (*Potamogeton pusillus*).

It was hoped that reductions in milfoil density associated with weevil release would bring about an increase in population size for these species. A sudden resurgence in size of the state endangered white-stemmed pondweed populations was noted at Saugany Lake in LaPorte County following a massive decline in milfoil density which was likely attributable to a native weevil population (Scribailo, personal observation). Recent correlative evidence indicates that both historical and current great declines observed in milfoil populations in lakes throughout the Midwest may be associated with native milfoil weevil populations (Creed, 1998).

Overlay maps are shown in Fig. 2-4 for GPS mapping of the three lakes. The only lake with mapping for all three years is Griffy Lake. Little Turkey lake was mapped for year one and three but not for year two because so little milfoil was present in the second year. Round Lake was mapped in the first and second years and only in some locations in the third year because herbicide permitting was accidentally allowed in the third year which destroyed all plants at transect two (west end of lake) and much of the beds at the east end of the lake.

In discussing the data from the study the best way to approach this is to summarize findings for each lake from each facet of the study together across years since this gives a better overall picture of trends. For clarity purposes, since discussing data from each transect individually does not alter the trend, data from all transects and biomass sampling has been treated together for each lake. After this discussion a brief overall statement can be made concerning the prospects of using milfoil weevils for biological control of Eurasian milfoil in Indiana.

At Round Lake aquatic plant beds are essentially present wherever water depth is less than 15 feet deep. Mapping for milfoil in Round Lake in the year 2000 (Fig.2) indicated that plants did not extend in a continuous bed completely across the lake although patches of plants were present in this zone. In 2001 beds surrounding the lake were fairly similar in extent to those found in 2000 with the exception that plants extended continuously across the lake in the shallow zone see green in Figure 2). This was also the case in 2002 although the extent of plants was greater as seen by the red lines across the center of the lake. The drought conditions and high summer temperatures of 2001-2002 that reduced lake levels could have been responsible for this trend. Vegetation was completely absent on parts of the eastern side of the lake in 2000 and again in 2002 where herbicide was applied.

Figure 5 shows biomass and AFDW (ash-free dry weight) values for the lakes. Note for Round Lake (RL) by year that despite some fluctuation dry weight biomass only varies slightly from year to year. Taking into account variations between quadrats these differences are not significant. Interestingly, AFDW values do vary more considerably. The comparatively high biomass but low AFDW for 2002 may be indicative of very fast growth rates which tend to produce plants with more intracellular space relative to actual organic matter deposition in cell wall materials. Note in Figure 6, which again shows biomass data, but this time in comparison to density data of plants per meter ^{2}, that density declined over the three years. This difference was significant between 2000 and 2001 but not so from 2001-2002. Biomass, as mentioned above, was not significantly different over the three-year period. Although it would be tempting to state that this decline was attributable to weevil activity Figure 7 indicates that this is a questionable correlation. In 2000 plant density and biomass were high and a significant population of both weevils and their larvae were present. Note at Round Lake that this assessment was done *prior* to weevil stocking since a native population of weevils was discovered there before the study began.

An examination of weevil damage at Round Lake indicated collapse of many stems due to a loss of buoyancy associated with larval burrowing through stem aerenchyma tissue. In the five quadrats assessed for weevil damage from each transect on average sixty percent of the plants showed signs of weevil damage typically as larval burrowing holes. Few adult weevils were found with typically only twenty percent of the plants having weevils in shoot tips. Larvae were more common as one would expect from the life cycle of the weevil and the comparatively short life span of adults with forty percent of plants having weevils. The presence of a native population of the weevils at Round Lake raises the question of why this was not investigated prior to a decision to release weevils at Round Lake. The use of a lake with an existing population of weevils for stocking makes it difficult to compare between lakes in this study.

In 2001 the weevil population of adults and larvae at Round Lake was less than one tenth of the previous year. It is certainly possible that weevil activity caused a reduction in biomass to the point that the following year plants were less productive. Nevertheless, given the fact that Eurasian milfoil was still extensive in 2001, one would expect, particularly since nine thousand weevils had been stocked just after our assessment, that the following year would show extensive populations of weevils. Furthermore, in 2002 no weevils or larvae were detected. The data from this study would tend to indicate that declines or fluctuations seen in Eurasian milfoil populations were more affected by adverse environmental conditions. This conclusion is borne out by a detailed examination of data from Little Turkey Lake and Griffy Lake.

Little Turkey Lake exhibited a complex distribution of milfoil beds in the 2000 survey (Figure 3). Unlike Round Lake, with the exception of milfoil there are few extensive beds of other aquatic plants present. Milfoil is limited somewhat in the extent of its growth by the substrate which is fine silt marl. Shiifting of this substrate due to wave action in shallow water along with its low nutrient availability reduce the potential size of milfoil populations compared to what is found in muckier soils like those found in parts of Round Lake. Milfoil tends to be found in protected bays and in open areas where islands block the impact of wave action. Unfortunately, no biomass data is available for 2000 for Little Turkey Lake since plants were inadvertently thrown out. Density data in Figure 7 does indicate that populations were extensive at the two transect locations (see Fig. 3). A striking feature of the data is the complete decline of milfoil in 2001 as shown by density data. Although no weevil counts were done the first year at little Turkey

Lake or at Griffy Lake (since there was no indication of pre-existing populations of weevils) plants were too few in 2001 to collect weevil data from at Little Turkey Lake. No mapped areas for milfoil are shown in Figure 3 since plants were too sporadic in distribution to map. Milfoil biomass increased dramatically in 2002 from almost nothing in 2001, although density of plants was not higher than that found at Round Lake. The extent of milfoil was so ubiquitous that it was impossible to map since the distribution encompassed almost the entire lake bottom from north to south. The extreme shallowness of the lake makes it possible for the milfoil to not be light-limited across most of the lake bottom.

No weevils were found on the plants at Little Turkey Lake in 2002 (Figure 7). It could be conjectured that the massive decline in milfoil from 2000 to 2001 was attributable to the weevil populations, which themselves then crashed because of lack of food materials, thus exemplifying a classic predator/prey dynamic population model. The absence of a resurgence of weevil numbers in 2002 would tend to argue against this as a likely explanation.

Mapping of milfoil at Griffy Lake over the three years indicates that the single greatest limiting factor on milfoil distribution is depth increase (figure 4). The steep slopes and drop-off rate in this lake limit milfoil to the shallow bays around the lake and also to a narrow band around the shoreline. Over the three-year study the most extensive bed of milfoil was found in the shallow bay east of the road crossing and at the location of the public access. This is indicated in the lowermost right side of the aerial photo. Since the entire bay was filled with milfoil this is not indicated in the map. The milfoil then extended under the bridge as far as is shown in the shallow areas west of the road in brown on Figure 4.

The eastern bay also has an extensive population of *Egeria densa* which is an exotic species not previously recorded from Indiana. This is a potential species of concern that is very aggressive and is a major problem aquatic weed in southern U.S. lakes. Over the three year-period the extent of milfoil declined progressively in this bay with the density of *Egeria* increasing proportionately. Figure 4 indicates an increase in milfoil abundance, particularly in 2002 where it was present as a narrow band extending completely around the lake (red in figure 4. It is important to note that the milfoil was intermixed with *Egeria* with the latter being the more dominant species in the zone bordering the reservoir dam to the northwest.

No biomass data was available for Griffy Lake for 2000 since an extremely early senescence caused plants to become uprooted and floating throughout much of the lake by the time of our sampling. We did still manage to get density data in some of the locations that were more intact. Note that density declined over the three years although the results for 2001 and 2002 are not significantly different. Biomass was lower in 2001 indicating the presence of smaller plants.

The question again is whether the decline from 2000 to 2001 was attributable to weevils. Our observations indicate that a massive early season algal bloom and subsequent senescence of that algae caused collapse of the milfoil beds. Most of the milfoil was dying by the time of our visit. Due to a shortage of weevils, stocking did not take place until later in the summer well after the senescence event. It is therefore unlikely that weevils were responsible for the decline by years-end of 2000. The reduced growth of milfoil in 2001 was certainly, in part, a product of the early season collapse the previous year. It is possible though, that weevils contributed to the maintenance of this decline by feeding on plant apical meristems in the early season, since data in Figure 7 does indicate the presence of a small number of larvae and adults. The number of weevils present is likely not different than that found in Round Lake for 2000 if the numbers are represented as a proportion of the number of plants examined. The complete absence of weevils at Griffy Lake in 2002 would also tend to argue against a conclusion that weevils were largely responsible for milfoil declines observed.

CONCLUSIONS

Data from this study is inconclusive concerning the possible role of weevils in reducing Eurasian milfoil populations at the three lakes in Indiana. The majority of evidence suggests that the fluctuations in milfoil populations were largely stochastic in nature and more attributable to unusual weather patterns during the years of the study. The only lake that offers some decent evidence that weevils may have been a contributing factor is data for Round Lake. This data set would have been more compelling if the best transect at the lake had not been accidentally herbicided in the third year.

Much of the problem in trying to draw conclusions relates to the short duration of the study and a plethora of confounding factors that make interpretations difficult, such as the presence of an existing weevil population at Round Lake and multiple herbicide applications during the study. Trying to run detailed experiments over many years in the face of a general attitude by lake residents that requires instant gratification with treatment methods is problematic. Certainly, if lake residents are interested in a "quick fix" for their aquatic weed problems, weevil stocking is not the answer.

This is not to say that weevil stocking is not recommended. Nevertheless, given the tremendous uncertainty that populations will establish, and the exorbitant price of the weevils, it is a large expense for most lake associations. A suggestion might be to transport plants from lakes with known populations of weevils to assist in the possible establishment of weevils without risking the expense for a control that may initially fail in the majority of lakes.

A realistic approach to lake management will involve integrating multiple methods together. DNR realizes, as do lake residents, that aquatic weed problems belie other watershed problems. If these watershed problems are aggressively pursued then lake weed management will be less of an insurmountable issue. A combination of methods involving sensible herbicide application combined with weevil stockings will probably offer the most effective control. Recent evidence from studies of water hyacinth control using a combination of these two methods seems to bear out this conclusion (Center et al., 1999). This study indicated that maintenance of water hyacinth populations at desirable levels without attempting eradication provided enough quality food materials that weevil populations were maintained at breeding levels that greatly assisted in control measures and in turn reduced the need for more extensive herbiciding.

It is hoped that the majority of lake residents realize that a minimum of forty percent coverage of lake bottom is desirable in terms of providing suitable habitat for invertebrates for fish foraging or as habitat for fish to escape predation and spawn. If milfoil herbicide application is done in a responsible manner only targeting areas most impaired for human activity this balance should be mainitained. Unfortunately some residents would prefer lakes that look like swimming pools, subsequently wondering where all the game fish have gone. Another factor that should be considered is the presence of many rare aquatic plant species in our lakes. It is important that efforts be made to properly document all rare species before herbicide permits are approved.

Fig. 1. Map of Indiana showing the location of the three lakes participating in the DNR weevil release study. Saugany Lake is also indicated which is the location of the first native population of weevils found in the state.

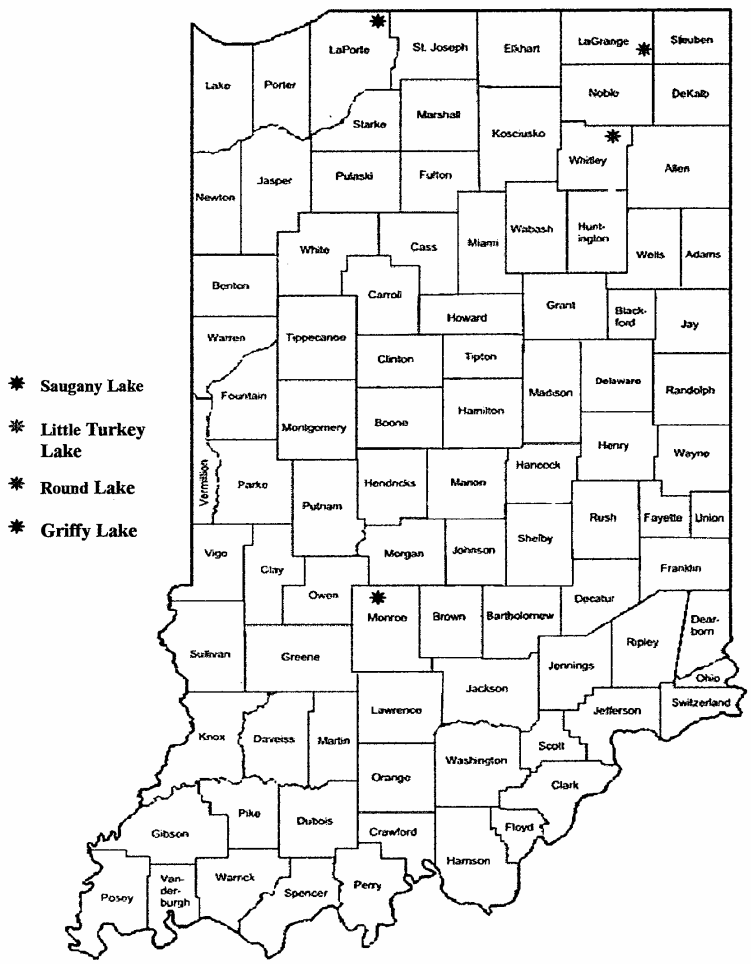


Table 1. Aquatic plant species list for Griffy Lake.

Latin Family	**Common Name**	**Species**
Boraginaceae	Common Forget-me-not	*Myosotis scorpioides*
Ceratophyllaceae	Common Coontail	*Ceratophyllum demersum*
Haloragaceae	Eurasian Water Milfoil	*Myriophyllum spicatum*
Hydrocharitaceae	Brazilian Elodea	*Hydrilla veticillata*
Lemnaceae	Small Duckweed	*Lemna minor*
	Star Duckweed	*L. trisulca*
	Giant Duckweed	*Spirodela polyrhiza*
Najadaceae	Slender Naiad	*Najas flexilis*
Nymphaeaceae	Yellow Water Lily	*Nuphar advena*
	White Water Lily	*Nymphaea odorata* subsp.
tuberosa		
Onagraceae	Marsh Purslane	*Ludwigia palustris*
Pontederaceae	Pickerel Weed	*Pontederia cordata*
Potamogetonaceae	Curly Leaf Pondweed	*Potamogeton crispus*
	Leafy Pondweed	*P. foliosus*
	Illinois Pondweed	*P. illinoensis*
	Small Pondweed	*P. pusillus*
	Sago Pondweed	*Stuckenia pectinata*
Typhaceae	Common Cattail	*Typha latifolia*
Zannichelliaceae	Horned Pondweed	*Zannichellia palustris*

Table 2. Aquatic plant species list for Round Lake.

Latin Family	**Common Name**	**Species**
Araceae	Arrow Arum	*Peltandra virginica*
Boraginaceae	Common Forget-me-not	*Myosotis scorpioides*
Ceratophyllaceae	Common Coontail	*Ceratophyllum demersum*
Haloragaceae	Eurasian Water Milfoil	*Myriophyllum spicatum*
Hydrocharitaceae	Slender Waterweed	*Elodea nuttallii*
	Eel Grass	*Vallisneria americana*
Iridaceae	Blue Flag	*Iris virginica*
Lemnaceae	Small Duckweed	*Lemna minor*
	Star Duckweed	*L. trisulca*
	Giant Duckweed	*Spirodela polyrhiza*
Lythraceae	Swamp Loosestrife	*Decodon verticillatus*
Najadaceae	Slender Naiad	*Najas flexilis*
Nymphaeaceae	Yellow Water Lily	*Nuphar advena*
	White Water Lily	*Nymphaea odorata* subsp.
tuberosa		
Onagraceae	Marsh Purslane	*Ludwigia palustris*
Pontederaceae	Pickerel Weed	*Pontederia cordata*
Potamogetonaceae	Large-leaved Pondweed	*Potamogeton amplifolius*
	Curly Leaf Pondweed	*P. crispus*
	Long-leaved Pondweed	*P. nodosus*
	Small Pondweed	*P. pusillus*
	White-stemmed Pondweed	*P. praelongus*

	Richardson's Pondweed	*P. richardsonii*
	Stiff Pondweed	*P. strictifolius*
	Flat-stemmed Pondweed	*P. zosteriformis*
	Sago Pondweed	*Stuckenia pectinata*
Typhaceae	Common Cattail	*Typha latifolia*

Table 3. Aquatic plant species list for Little Turkey Lake.

Latin Family	Common Name	Species
Ceratophyllaceae	Common Coontail	*Ceratophyllum demersum*
Haloragaceae	Eurasian Water Milfoil	*Myriophyllum spicatum*
		Myriophyllum heterophyllum
Hydrocharitaceae	Slender Waterweed	*Elodea nuttallii*
	Eel Grass	*Vallisneria americana*
Lemnaceae	Small Duckweed	*Lemna minor*
	Giant Duckweed	*Spirodela polyrhiza*
Najadaceae	Slender Naiad	*Najas flexilis*
	Southern Naiad	*N. guadalupensis*
Nymphaeaceae	Yellow Water Lily	*Nuphar advena*
	White Water Lily	*Nymphaea odorata* subsp.
Tuberosa		
Pontederaceae	Pickerel Weed	*Pontederia cordata*
Potamogetonaceae	Curly Leaf Pondweed	*Potamogeton crispus*
	Illinois Pondweed	*P. illinoensis*
	Sago Pondweed	*Stuckenia pectinata*

FIG. 5. BIOMASS (FRESH WEIGHT) AND ASH FREE DRY WEIGHT PER M^2 GIVEN BY SITE AND YEAR

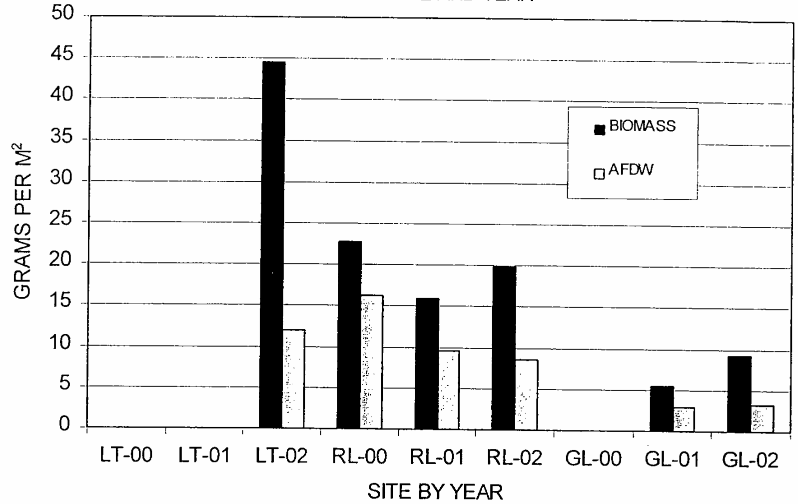


FIG. 6. BIOMASS (FRESH WEIGHT) AND PLANT DENSITY GIVEN BY SITE AND YEAR.

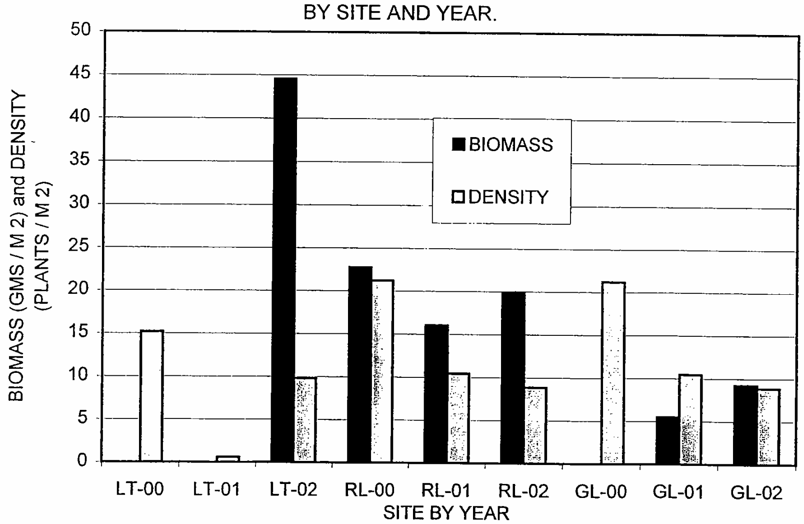
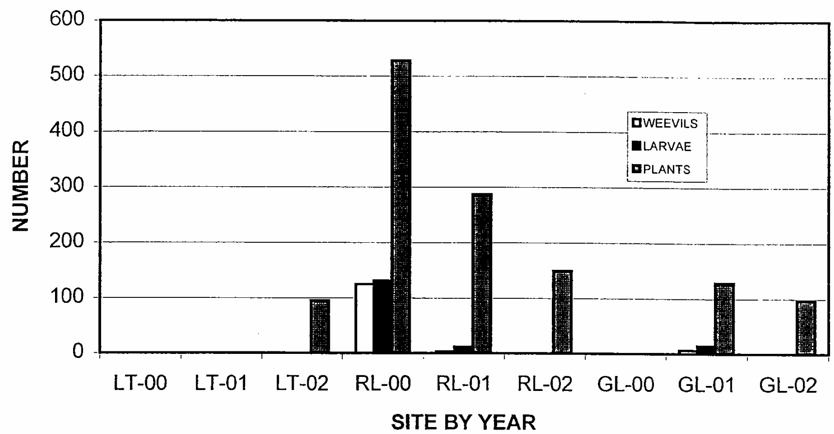


FIG.7. NUMBER OF WEEVILS (ADULT AND LARVAE) AND NUMBER OF PLANT EXAMINED GIVEN BY SITE AND YEAR.



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